THE PHYSIOLOGICAL DIVISION OF LABOUR IN THE SALIVARY GLANDS OF *ONCOPELTUS FASCIATUS* (DALL.) (HETEROPTERA : LYGAEIDAE)

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Summary

The physical and chemical characteristics of the contents of the different lobes of the salivary glands of *O. fasciatus* are compared with the characteristics of the two types of salivary secretion: namely the solid "stylet sheath" and the liquid "watery saliva". It is concluded that the anterior lobe contributes most of the sulphydryl groups that form the disulphide bonds in the sheath material, but that the lateral lobe provides most of the bulk of the sheath; that the posterior lobe contributes the digestive enzymes found in the watery saliva; that the accessory gland secretes water that can dilute the watery saliva; and that the accessory gland also provides the polyphenol oxidase that is sometimes found in the watery saliva and is always secreted along with the sheath material. Surgical isolation of the accessory gland gave no positive information on the function of the polyphenol oxidase.

I. Introduction

Many phytophagous Hemiptera are now known to produce two types of salivary secretion, one that solidifies to form the "stylet sheath", and a watery saliva that does not solidify and may contain digestive enzymes (Miles 1958, 1959*a*; Saxena 1963). Yet there is still doubt both as to the function of these secretions (Miles 1959*b*; Saxena 1963) and as to their origin (Salkeld 1960; Miles 1960*b*).

A comparison of the studies on the salivary physiology of *Oncopeltus* by Bronskill, Salkeld, and Friend (1958), Salkeld (1959, 1960), and Miles (1960*b*) is made difficult by the use of different conventions in the naming of the parts of the complex salivary apparatus (Fig. 1). The confusion probably arises because, in the nomenclature used by Baptist (1941) and followed by Nuorteva (1956) and Miles (1960*b*), the "lateral" lobe actually lies nearest to the midline inside the insect; and Bronskill *et al.* have used the term "lateral" for the bifid lobe in *Oncopeltus* that does indeed lie lateral to the midline. Nuorteva, however, called this bifid lobe the "posterior" lobe because it is anatomically homologous with the lobes that Baptist called "posterior" in other lygaeids; and, as this paper shows, it is also functionally homologous with the posterior lobe in pentatomids, in which a lateral lobe is lacking.

Bronskill *et al.*, Salkeld, and Miles agree on the mainly proteinaceous nature of the contents of the principal gland, and Salkeld (1960) and Miles (1960*b*) agree on the complex nature of the contents of the anterior lobe and its likely contribution to the sheath material (i.e. the solidifying component of the saliva). But although these authors also find enzymes, lipids, and carbohydrates in other parts of the apparatus, they do so in seemingly irreconcilable combinations.

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Subsequent studies on *Eumecopus*, a pentatomid (Miles 1961, 1964*b*), demonstrated that, in this insect, in which the principal salivary glands have only two lobes, the anterior lobe contains the precursor of the sheath material; the posterior lobe contains a water-miscible liquid; and the accessory gland elaborates a polyphenol oxidase that can also be found in the watery saliva. In *Eumecopus*, the precursor of the sheath material is a lipoprotein that is rich in sulphydryl groups: it gels readily, with the formation of both hydrogen bonds and disulphide bonds, the latter resulting from oxidation of the sulphydryl groups.

In the light of this information, a reappraisal was made of the salivary physiology of *Oncopeltus*. In an attempt to minimize artefacts during investigation of the origin of salivary enzymes, all observations on enzyme activity were made on live cells, or on the unfixed contents removed from the lobes of the freshly dissected glands, or on freshly secreted saliva. The nomenclature used in this paper for the salivary lobes follows the convention of Baptist and Nuorteva.

II. Materials and Methods

(*a*) *Source of Insects*

The insects were obtained from a colony kept in the Department of Entomology and Parasitology of the University of California, Berkeley. They were maintained on milkweed seed and water.

(*b*) *Method of Obtaining Sheath Material*

Unstretched Parafilm M^{R}, covering and in contact with 10–15% aqueous sucrose, was left overnight in a populous colony of the insects. The sheath material was excised from the film the following day.

(*c*) *Method of Observing the Reactions of Salivary Secretions*

The freshly dissected glands were rinsed in the salt solution of Martignoni and Scallion (1961) and placed under paraffin oil with a drop of reagent. The lobe in question was then punctured with a fine needle and the ensuing reaction observed under the microscope. The reactions of sheath material, gelled materials from the principal glands, and drops of watery saliva were observed similarly.

Sulphydryl groups were detected using a nitroprusside reagent (Pearse 1953). On solid materials, tests were also made using dihydroxydinaphthyldisulphide in conjunction with sulphydryl blockade by *N*-ethyl maleimide and disulphide reduction by thioglycollate, as described by Pearse.

The pH of the secretions was determined by mixing them with small amounts of 0·4% aqueous solutions of bromocresol purple, cresol red, and thymol blue.

(*d*) *Extraction of Lipids*

The contents of the principal gland were gelled in glycerol, and their lipids extracted as described by Miles (1961), except that the gels were rinsed in borate buffer, pH 10, which helped detach any residual adherent tissue, and extraction was

done in a 2 : 1 mixture of chloroform and methanol as described by Folch, Lees, and Sloane Stanley (1957).

(*e*) *Demonstration of Enzymes*

In order to investigate the localization of enzymes in the salivary glands, the lobes were first separated as described by Miles (1959*b*, p. 277) and ruptured in 0·05 ml buffer: 40 anterior lobes were used at a time, or 20 lateral lobes or posterior lobes. To these preparations was added 0·05 ml of a substrate, and the mixture was incubated for 1–2 hr at 25°C. Tests were made for amylase and esterase. For amylase, the buffer was veronal–acetate, pH 6·0 (Pearse 1953), and the substrate was the same buffer containing 1% soluble starch and 1% sodium chloride; after 1 hr, 0·05 ml 1% iodine in aqueous 2% potassium iodine was added (undigested

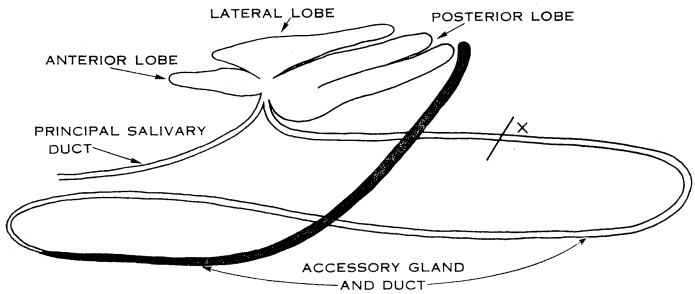

Fig. 1.—Salivary gland of *Oncopeltus fasciatus* (semi-diagrammatic) showing the part of the accessory gland that produces the salivary polyphenol oxidase in black. The approximate position where the duct of the accessory gland was cut in operations (see text) is at *X*. Bronskill, Salkeld, and Friend (1958) refer to the bifid posterior lobe as the "lateral lobe", and to the lateral lobe as the "posterior lobe".

starch gives a blue-black colour with iodine, and digestion causes a progressive reddening and diminution of this colour). For esterase, the buffer was made by titrating 1N sodium acetate containing 50 mg/l phenol red with 0·1N HCl until the solution was just distinctly pink, and the substrate was made in the same way except that the solution was saturated with tributyrin before titration (digestion of the tributyrin liberates butyric acid which destroys the pink coloration). Controls were provided by keeping the enzyme preparation and substrate apart until the time of comparison with the treatment.

Amylase was also detected in individual glands by rupturing single lobes into 1 μl of substrate under paraffin oil, and then adding 1 μl of iodine solution after incubation for 1–2 hr at 35°C; but care had to be exercised in interpreting these results because reducing activity such as is found in the contents of the anterior lobe (see below) is capable of reducing iodine and thus of discharging the colour of any starch–iodine complex formed initially. Watery saliva was similarly tested for amylase by incubation of 1-μl lots of saliva with 1 μl of buffered starch solution either under paraffin or in 0·5 ml stoppered tubes.

Polyphenol oxidase was demonstrated as described previously (Miles 1964*a*).

(*f*) *Demonstration of Amino Acids*

Lobes were separated and their contents mixed with buffer as described in Section II(*e*) above, except that borate buffer, pH 10, was used. The mixtures were analysed by unidimensional chromatography on Whatman No. 1 filter paper, using n-butanol–acetic acid–water (77 : 6 : 17 v/v) as solvent and 1% ninhydrin in ethanol containing 0·25% triethylamine to reveal amino acids (Hackman and Lazarus 1956).

TABLE 1

ABILITY OF CONTENTS OF THE SALIVARY GLANDS AND OF THE SALIVARY SECRETIONS OF *ONCOPELTUS* TO FORM GELS WHEN DISCHARGED INTO VARIOUS REAGENTS

Reagent	Contents of Principal Gland			Salivary Secretions	
	Anterior Lobe	Lateral Lobe	Posterior Lobe	Sheath Material	Watery Saliva
Glycerol	+	+	+*	+	−†
Saline	+	+	−	+	−
1% ascorbic acid	+	−	−	+	−
10% ascorbic acid	−	−	−	+‡	−
8M urea	−	−	−	+‡	−

* Gel formed in glycerol but dispersed when placed in water and dissolved completely when placed in saline.

† As collected directly from the mouthparts this secretion left hardly any deposit when it evaporated, and it was completely miscible with glycerol.

‡ Gel formed but it was spongy and fragile.

(*g*) *Surgical Isolation of the Accessory Gland*

Adults were anaesthetized in water-saturated CO_2 and were stretched out under Martignoni and Scallion's (1961) solution. A sharp needle was used to penetrate the intersegmental membrane behind the metanotum on one side of the midline, and a fine hook was used to fish out the proximal part of the tubular accessory gland, which was then cut (see Fig. 1). The process was then repeated for the other side. In control operations, the ducts were fished out and then replaced uncut.

III. RESULTS

(*a*) *Formation of Gels by Contents of the Lobes of the Principal Gland and by the Salivary Secretions*

The ability of the sheath material to gel as it was discharged by the insect into various reagents (Table 1) was tested by allowing the insects to probe through Parafilm into a solution of the reagent in 15% aqueous sucrose. The insects tended to secrete sheath material where the Parafilm overlaid the edge of the container, but this difficulty was partly overcome by allowing a drop of alcoholic extract of milk-

weed seed to evaporate on the Parafilm away from the edges; the deposit so formed seemed to act as a phagostimulant.

The sheath material was found to gel in reagents that prevented gelling of any of the contents of the principal gland. Moreover the contents of the different lobes differed between themselves in their ability to gel, e.g. the contents of the anterior lobe formed a gel in a concentration of reducing agent (ascorbic acid) that prevented gelling of the contents of the lateral lobe.

The contents of all the lobes of the principal gland were completely miscible with one another when the gland was ruptured under paraffin oil. They showed no tendency to gel when mixed in this way and the mixture thus formed did not gel any more readily when aqueous reagents were added to it under paraffin oil than did the contents of the lateral lobe alone.

(*b*) *Lipids in the Salivary Secretions*

The weight of stable gel obtained per lobe from the anterior and lateral lobes was 2·5 and 20 μg respectively, whilst the amount of lipid extracted from the gelled contents was 0·02 and 2·5 μg per lobe respectively. These results are means for 100 glands. Thus the amount of lipid extractable from gelled contents of the lobes was approximately 10% of the dry weight of either. After extraction, these gels swelled more readily and dissolved more quickly in reagents. Sheath material also contains lipids (Miles 1960*a*) and was similarly more readily attacked by reagents after its lipids had been extracted.

Since no stable gel formed in the posterior lobe, and the watery saliva proved very dilute, it was difficult to determine whether any lipids occurred in either of them; but since both were completely miscible with saline, it is very likely that they contain little or no lipid.

(*c*) *Some Chemical Characteristics of the Contents of the Principal Gland*

The secretion of the anterior lobe had a particularly high content of sulphydryl groups (Table 2), and a high concentration of ascorbic acid was required to prevent gelling. Hence it seemed likely that the formation of disulphide bonds by the oxidation of sulphydryl groups contributed to the formation of gels when gelling occurred in the contents of this lobe. This conclusion was supported by the fact that, after the sulphydryl groups had been blockaded with *N*-ethyl maleimide, the gel that now formed was soluble in urea (Table 3) and was presumably stabilized mainly by hydrogen bonds.

The behaviour of the contents of the lateral lobe was seemingly anomalous when compared with the contents of the anterior lobe. The former appeared to contain a lower concentration of sulphydryl groups, and, consistent with this, a lower concentration of ascorbic acid prevented gelling, yet the contents of the lateral lobe gelled *during* incubation in *N*-ethyl maleimide and this gel was not soluble in urea. Conceivably substances other than protein may have been involved in its formation: although carbohydrates were not detected on chromatograms of hydrolysates (e.g. with hydrogen aniline phthalate or urea–HCl reagents), more recent studies on

Eumecopus (unpublished), a pentatomid, have indicated that, in the latter insect, small amounts of carbohydrate are incorporated in gels of its salivary secretions. Nevertheless, the failure of prior treatment with *N*-ethyl maleimide to affect the properties of the gelled contents of the lateral lobe in *Oncopeltus* is most probably due to the ease with which hydrogen bonds formed in the secretions of this lobe.

TABLE 2

SUMMARY OF VARIOUS CHEMICAL PROPERTIES OF THE CONTENTS OF THE SALIVARY GLANDS AND OF THE SALIVARY SECRETIONS OF *ONCOPELTUS*

Properties are semiquantitative estimates relative to bulk: −, not detected; ±, trace; +, ++, +++, ++++, degree of strength; 0, test not feasible or not applicable

Chemical Property Tested	Contents of Principal Gland			Salivary Secretions	
	Anterior Lobe	Lateral Lobe	Posterior Lobe	Sheath Material	Watery Saliva
Sulphydryl groups	++++	++	++	+	0
Soluble protein*	++	+	+++	0	±
Free amino acids†					
Alanine and below	++	+	+++	0	±
Above alanine	+	−	±	0	−‡
pH	>7·2	<7·2	>7·2	≃5·8§	⩾8·0‖
Enzymes					
Amylase	−	−	+	0	+
Esterase	−	−	+	0	0¶

* Compounds that were soluble in water and reacted with ninhydrin but remained at the origin of paper chromatograms are assumed here to be mostly protein.

† As detected on paper chromatograms run in butanol–acetic acid–water (77 : 6:17 v/v).

‡ Experiments to be described in a further paper showed that a given amino acid could be made to appear or increase in concentration in the watery saliva by injection of the compound into the haemolymph.

§ Miles (1960*a*).

‖ Miles (1965).

¶ See discussion in Section III(*c*) on the dilute nature of this secretion as collected experimentally.

It was found that almost any treatment of the glands that resulted in the death of the cells caused the immediate formation of a skin of denatured protein around the bulk of the contents of the lateral lobe, and denaturation thereafter spread throughout the whole mass. And a ready formation of hydrogen bonds in this way would both tend to protect sulphydryl groups from reagents (Swan 1957) and cause them to form disulphide bonds before the sulphydryl groups could be blockaded.

Amylase and esterase were found in the posterior lobe only of the principal gland. Amylase was also found in the watery saliva, but, although esterase is known to be discharged by these insects during feeding (Feir and Beck 1961), this enzyme was not detected in any of the salivary secretions that were collected from the insects' mouthparts. The failure to do so was probably due to (i) the very dilute

nature of the watery saliva that could be collected from the insect, and (ii) the relative insensitivity of the test for esterase. It was found that *Oncopeltus* produced watery saliva readily if the insects had been sprayed with a mist of water just beforehand, but the saliva collected in these circumstances proved very dilute indeed: it showed not even amylase activity, failed to react with ninhydrin, and left hardly any detectable deposit when it evaporated. Watery saliva was also obtainable from "unsprayed" insects, although in very much smaller quantities: such saliva did react with ninhydrin, and showed weak amylase and polyphenol oxidase activity, but the tests for these two enzymes were considerably more sensitive than that employed for esterase.

TABLE 3

SOME CHARACTERISTICS OF DRIED, GELLED CONTENTS* OF SALIVARY GLANDS AND OF SHEATH MATERIAL OF *ONCOPELTUS* WITH RESPECT TO SWELLING AND DISSOLUTION

Reagent	Anterior Lobe	Lateral Lobe	Sheath Material
Water	Swells	Swells slightly	Swells very little
8M urea	Swells	Swells slightly	Swells slightly
Urea–sodium sulphide†	Dissolves	Dissolves	Dissolves
8M urea after glands incubated in *N*-ethyl maleimide‡	Dissolves	Gel forms during incubation; does not dissolve in urea	—

* After immersion in glycerol.

† Freshly prepared 8M urea saturated with sodium sulphide.

‡ Glands placed overnight in 0·01M *N*-ethyl maleimide at 4°C to blockade sulphydryl groups.

(*d*) *Salivary Polyphenol Oxidase*

Freshly secreted sheath material from normal insects always showed polyphenol oxidase activity: it reacted with dihydroxyphenylalanine (DOPA) within 1 hr at 25°C, and was prevented from doing so by 0·005M cyanide or phenylthiourea. Frequently, a particularly strong reaction occurred in secretion remaining in the stylet channel that runs through the centre of the stylet sheath. This secretion may have been watery saliva, and 1-μl quantities of the watery saliva from "unsprayed" insects darkened a 5-μl drop of 0·2% DOPA at pH 7·4 within 1 hr at 35°C.

When whole glands were incubated with DOPA, the distal part of the accessory gland gave a strong reaction, but the proximal loop, i.e. the part that travels posteriorly from the hilus of the principal gland to form a loop near the junction of the thorax and abdomen (see Fig. 1), gave little or no reaction within 2 hr at 35°C. No other part of the gland appeared to react with DOPA, and the lack of activity in the principal salivary duct was particularly noteworthy in view of the activity shown by this part of the salivary apparatus of other Pentatomorpha, including

lygaeids (Miles 1964*a*). Cyanide and phenylthiourea inhibited the reaction of the accessory gland.

(*e*) *Isolation of the Accessory Gland*

In an initial series of operations, up to 80% of the insects in which the ducts of the accessory glands had been cut survived for a week or longer; but the recovery rate after operations made over the succeeding 2 months dropped to 40%. No reason for this was apparent, and special precautions to keep instruments and solutions sterile did not seem to improve survival.

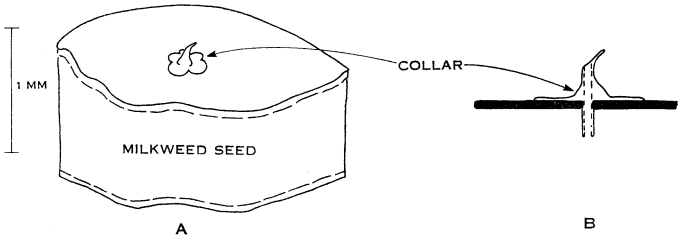

Fig. 2.—*A*, sheath material deposited on a milkweed seed by *Oncopeltus fasciatus* (semi-diagrammatic). *B*, diagrammatic cross-section through the point of insertion of the stylets into a milkweed seed, showing how the sheath material is continued as only a short stump on the inner side of the testa.

The insects that recovered began to feed on milkweed seeds within 12 hr of the operation, and external collars of sheath material were recovered from the seeds (Fig. 2*A*). Control insects, in which the ducts of the accessory glands had been exposed but replaced uncut, showed a temporary lowering of the polyphenol oxidase activity in their sheath material, but this returned to normal within a day or two. On the other hand, the sheath material deposited by the insects that had had the duct cut on both sides showed no detectable polyphenol oxidase activity. Despite this, their sheath material did not seemingly differ otherwise from normal; it was not noticeably affected to any greater extent by urea–sodium sulphide or by pepsin (Miles 1960*a*) or pancreatin (Miles 1964*b*). The operated insects were able to feed apparently normally on milkweed seed and on the phloem of bean plants (*Vicia faba* F.), leaving behind a sheath that was possibly a little bulky but otherwise indistinguishable from normal.

Of the survivors that had moulted only a few days before the operation, many went on living for over 3 weeks. After a week, their sheath material began to show traces of polyphenol oxidase activity once more. Glands dissected out at this time showed a slight reaction in the remaining stump of the duct of the accessory gland and in the principal salivary duct, and these were probably the source of the activity. The part of the accessory gland that had been severed showed no direct connexion with the principal gland; but although it had a diminished polyphenol oxidase activity, this nevertheless still exceeded the activity of the ducts attached to the principal gland.

(*f*) *Secretion of Saliva into Milkweed Seeds*

When *Oncopeltus* feeds on stems, a complete stylet sheath is formed (Miles 1959*b*); whereas, when the insect fed on milkweed seeds, a bulky collar of material was first built up on the testa and, once the testa had been punctured, the sheath continued as a relatively thick stub that penetrated only a very short way into the interior of the seed (Fig. 2*B*). Nevertheless, movements of the insect's head and the elbowing and straightening of the rostrum indicated that the stylet bundle worked its way back and forth within the seed far beyond the end of this abbreviated sheath. After about $\frac{1}{2}$ hr of uninterrupted feeding, the interior of the seed had been thereby reduced to a watery pulp that could be expressed through the central canal of the sheath collar by squeezing the seed (always providing the insect had been removed before it had had time to suck it all out). Since the contents of the seed were originally quite dry and firm, this conversion must have been brought about by the watery saliva, aided, no doubt, by the macerating action of the stylets (Saxena 1963).

IV. Discussion

(*a*) *Origin of the Watery Saliva and the Digestive Enzymes of the Saliva*

The watery saliva that could be collected from plant bugs by manipulation of the rostrum (Day and Irzykiewicz 1954) was found to be much more dilute than any of the contents of the lobes of the principal gland, and the presence sometimes of polyphenol oxidase (Miles 1964*a*) points to the accessory gland as at least one origin of this saliva. Very dilute saliva can flow copiously at times, especially when the insects are subjected to high humidity, and the accessory gland itself has no lumen in the Pentatomorpha. Thus it follows that the accessory gland (or some other part of the duct system) must rapidly secrete water from the haemolymph. Indeed Goodchild (1966) believes this to be one of the basic functions of the accessory gland throughout the Heteroptera.

Nevertheless, digestive enzymes can also be present in the watery saliva, and these clearly originate in the principal gland. At least four digestive enzymes — an amylase, an invertase, a proteinase, and an esterase — have been demonstrated in the principal gland (Bronskill, Salkeld, and Friend 1958; Salkeld 1959) and the discharge of these enzymes during feeding has been confirmed (Feir and Beck 1961). The amylase at least has now been demonstrated in the watery saliva as such; but the results of Bronskill *et al.* pose a formidable problem, for although these authors state that the posterior lobe* elaborates most of the salivary amylase and invertase, they claim that the lateral lobe is the sole source of the proteinase and esterase. But the lateral lobe is an obvious source of the sheath material; thus, if Bronskill *et al.* are correct, the salivary proteinase and esterase are secreted only along with the sheath material; and, since *Oncopeltus* secretes very little sheath material within a milkweed seed, it would follow that the pulp into which the insect turns the interior of the seed during feeding must be produced without the aid of these enzymes.

* Actually, Bronskill *et al.* refer to this bifid lobe as the "lateral lobe", and to the lateral lobe as the "posterior lobe".

No doubt a combination of stylet action and the discharge of water from the accessory gland is essential for the formation of the pulp within the seed, much as Saxena (1396) describes in relation to the feeding of *Dysdercus* on cottonseed; but it would be surprising if a major portion of the digestive enzymes elaborated by the salivary system were not also involved. The interpretation of the role of salivary enzymes during the feeding of *Oncopeltus* presents no problem, however, if the finding of Bronskill *et al.* of digestive enzymes in the contents of the lateral lobe is mistaken; and certainly I have been unable to confirm their results with respect to esterase, which I find, together with the amylase, only in the posterior lobe. Salkeld later made some elegant histochemical analyses of the contents of the salivary glands, and it is unfortunate that she does not state which convention she is following in the naming of the principal lobes; from her photographs (1959) and the results of her tests for lipids (1960) it seems possible that, in these papers, she used the convention established by Baptist and Nuorteva; and hence that she also finds the esterase in the posterior lobe alone.

If the digestive enzymes of the salivary glands all originate in the posterior lobe, then it is possible to assign a more significant role to the salivary enzymes during feeding. It would follow that the watery saliva ejected during feeding comes mainly from the posterior lobe, and that all the digestive enzymes are brought into play simultaneously and independently of whether or not a stylet sheath is being laid down. This hypothesis is supported by observations reported here and previously (Miles 1960*b*) on the origin of enzymes in the salivary glands and is in accord with observations of the feeding of *Oncopeltus* on artificial substrates (Miles 1959*b*); it is also supported by determinations of the pH of the salivary secretions, for the watery saliva discharged during feeding has a pH of about 8, which corresponds with the pH of the contents of the posterior lobe but not the lateral lobe. Nevertheless, the secretion from the posterior lobe can apparently be diluted with water secreted by the accessory gland; and, in special circumstances (e.g. manipulation of the insects in very humid conditions), the discharge of the accessory gland seemingly replaces the saliva elaborated in the posterior lobe.

(*b*) *Origin of the Sheath Material and its Chemical Stability*

If the anterior lobe and lateral lobe are unlikely to be the sources of the watery saliva because their contents gel readily, then, for the same reason, it is likely *a priori* that they go to form the solid component of the salivary secretions, namely the sheath material. Their ability to form both disulphide bonds (especially the contents of the anterior lobe) and hydrogen bonds (especially the contents of the lateral lobe) corroborate this. The contents of the lateral lobe are far bulkier than the contents of the anterior lobe; and of all the contents of the principal glands, the contents of the lateral lobe have a pH that best corresponds to that of the sheath material.

The production by *Oncopeltus* of sheath precursor from two separate lobes of the salivary gland, whereas the same function is performed by the one (anterior) lobe in pentatomids, could well be a specialization related to the energy required to prevent reaction between the sulphydryl groups of the precursors. In *Oncopeltus*,

the greatest concentration of sulphydryl groups is isolated in the anterior lobe in a small quantity of protein that forms relatively few hydrogen bonds; and presumably this part of the salivary apparatus is highly specialized to maintain reducing conditions. Meanwhile the bulk of the protein of the sheath is kept under conditions that are specialized to prevent the formation of hydrogen bonds. Nevertheless, gelling of the precursors is prevented not only in the lobes themselves, but also during admixture and discharge of the secretions through the ducts and salivary canal; and this probably accounts for the sulphydryl (and hence reducing) content that all the lobes of the principal gland show to some extent.

TABLE 4

SUMMARY OF DISTINGUISHING CHARACTERISTICS OF PARTS OF THE SALIVARY GLANDS AND OF THE SALIVARY SECRETIONS OF *ONCOPELTUS*

	Physical Characteristics	Chemical Characteristics	pH
Contents of:			
Anterior lobe	Gels in water	Forms –SS– bonds	$>7\cdot2$
Lateral lobe	Gels in water	Forms hydrogen bonds	$<7\cdot2$
Posterior lobe	Miscible with water	Contains digestive enzymes	$>7\cdot2$
Accessory gland	—	Secretes polyphenol oxidase and probably water	?
Salivary secretions			
Sheath material	Solid	Contains –SS– and hydrogen bonds and polyphenol oxidase	$5\cdot8$
Watery saliva	Liquid	Contains digestive enzymes and polyphenol oxidase	$\geqslant8\cdot0$

The sheath material has greater chemical stability than either of its precursors when these are gelled within the salivary glands (and this is also true when only one precursor is elaborated, as in the Pentatomidae). An explanation for this has already been advanced (Miles 1964*b*): that the newly secreted sheath material is intimately mixed with the polyphenol oxidase that originates in the accessory gland; and that on exposure to air or dissolved oxygen, the enzyme acts on a polyphenolic substrate in the saliva to produce quinones that in turn form covalent linkages within the sheath material, very possibly through its sulphydryl groups. Nevertheless it has been reported here that the isolation of the part of the accessory gland that produces nearly all the salivary polyphenol oxidase did not affect the stability of the sheath, although it did abolish the reaction of the sheath with DOPA. This result throws considerable doubt on the involvement of the salivary polyphenol oxidase with the stabilization of the sheath. It is possible that, in these experiments, the enzyme was still secreted in the saliva of the operated insects in amounts that were undetected but sufficient to consolidate the sheath material; and the reappearance of detectable polyphenol oxidase activity in the sheath material of the insects a week after the operation perhaps argues in favour of this view. Nevertheless, there can now be no doubt that the amount of the enzyme secreted by the insects is greatly in excess

of any possible requirement for the stabilization of the sheath; and the primary function of this prominent feature of the saliva of many phytophagous Hemiptera still awaits a satisfactory explanation.

(*c*) *Salivary Function during Feeding*

A clear division of labour appears in the salivary glands of Pentatomorpha; and, in *Oncopeltus* (see Table 4), this would seem to be as follows: the anterior lobe contributes a sulphydryl-rich protein to the sheath material, and when this precursor gels on its own, it forms mainly disulphide bonds; the lateral lobe contributes the bulk of the sheath material and the precursor from this lobe gels by forming mainly hydrogen bonds; the posterior lobe produces most if not all of the digestive enzymes of the watery saliva; and the accessory gland produces a polyphenol oxidase and is probably responsible for rapid discharges of water in the saliva. How these different elements fit together during the complex feeding process (Miles 1959*b*), however, still requires elucidation. Clearly, when sheath material is secreted, it is mixed with the polyphenol oxidase from the accessory gland (for the reaction of the sheath with polyphenols can be abolished by severing the accessory gland); but it also seems likely that the accessory gland contributes a very dilute secretion that may be discharged without any sheath material. It remains to be demonstrated unequivocally whether the secretion from the posterior lobe (1) is sometimes discharged with the sheath precursors (and hence that the entire apparatus can discharge simultaneously, for the sheath precursors are themselves discharged with the secretion of the accessory gland); (2) is always diluted with water secreted by the accessory gland; and (3) may be discharged entirely on its own.

The function of the sheath itself is probably twofold. As Saxena points out, it serves to steady the stylets when they attempt to penetrate a hard surface; but the continuation of a sheath all the way down through soft plant tissues to the phloem vessels is good evidence for Mittler's (1954) interpretation that the sheath prevents the escape of liquids into surrounding tissues during feeding (for a discussion of this, see Miles 1959*b*).

Probably the most interesting problem concerning the feeding of the Pentatomorpha that remains to be solved is the function of the salivary polyphenol oxidase, for it is elaborated in significant amounts, by a specialized part of the salivary apparatus, and the enzyme itself is capable of producing highly reactive products (quinones).

V. Acknowledgments

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VI. References

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